

# The BAFF inhibitor AMG523 blocks adhesion and survival of human multiple myeloma cells in the bone marrow microenvironment: clinical implication

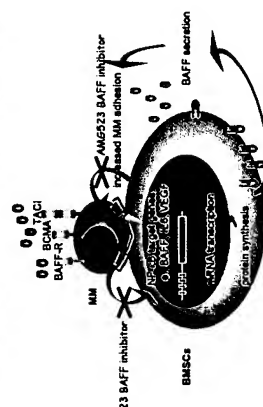
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## ABSTRACT

We previously identified a role of B-cell activating factor (BAFF), a member of the tumor necrosis factor superfamily, in the adhesion and survival of MM cells in the BM. BAFF is secreted by stromal cells and is a key component of the bone marrow microenvironment. In this study, we investigated the effect of BAFF inhibition on MM cell survival in the BM. We used a novel BAFF inhibitor, AMG523, to block BAFF signaling in MM cells. AMG523 treatment significantly reduced MM cell survival in the BM, as measured by colony formation and cell viability assays. This effect was associated with increased apoptosis and decreased proliferation. Furthermore, AMG523 treatment significantly reduced MM cell adhesion to bone marrow stromal cells, as measured by adhesion assays. These findings suggest that BAFF signaling is critical for MM cell survival and adhesion in the BM, and that inhibition of BAFF signaling may be a novel therapeutic approach for MM.

## BAFF Mediates MM Adhesion-Induced Growth and Survival



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## Aims of this study of AMG523 in MM

- Block BAFF-induced signaling in MM
- Direct or indirect inhibition of growth & survival of MM cells alone or with BAFF stimulation (BMSCs (adherent vs. peritoneal))
- Effect on drug-induced apoptosis in MM cells
- Effect on MM adhesion to BMSCs induced by BAFF
- In combination with conventional and novel therapies in MM

## DISCLOSURE STATEMENT

All authors, except Dr. Jiangchun Xu, in the abstract have no relevant conflicts of interest to disclose.

Dr. Jiangchun Xu is a paid employee of AMGEN.

## RESULTS

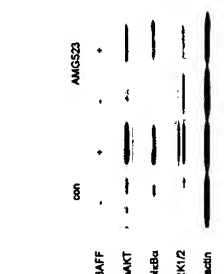


Figure 1. BAFF inhibitor AMG523 blocks BAFF-induced signaling in MM cells. MM cells were treated with AMG523 (100 ng/ml) for 2 h and pretreated with 20 ng/ml of anti-BAFF antibody (anti-BAFF) for 7 minutes. Cell lysates were immunoblotted with anti-pAKT, -pERK1/2, and -pAkt. Actin was used as a loading control.

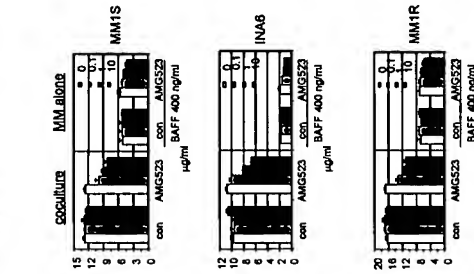


Figure 2. Effect of AMG523 on BAFF-induced adhesion. MM1S, INA6, and MM1R cells were incubated with BAFF (200 ng/ml) or with BAFF + AMG523 (100 ng/ml) for 2 h. Adherent cells were quantified by a fluorescence plate reader. (B) AMG523-inhibited adhesion induced by BAFF was performed using a 96-well plate. Data were normalized with control without BAFF or without AMG523.

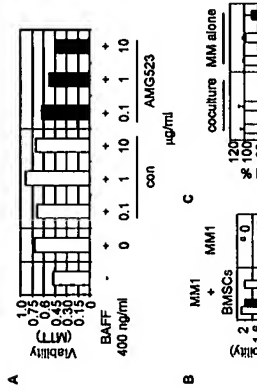


Figure 3. BAFF and adhesion-induced survival in MM cells. (A) CD138-positive MM cells were incubated with BAFF (200 ng/ml) for 3 days followed by MTT assay to measure cell viability. (B) Patient MM cells were incubated with BAFF (200 ng/ml) for 3 days followed by MTT assay for cell viability. (C) Percentages of survival from 6 patient MM cells cultured with BAFF in the presence of AMG523 or con.

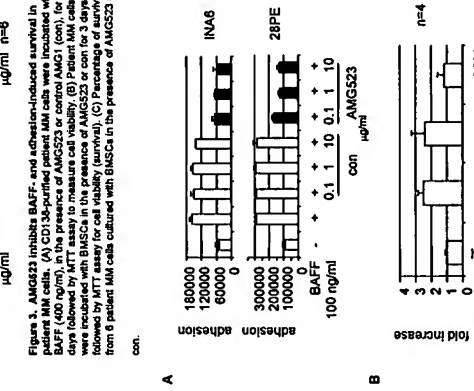


Figure 4. AMG523 blocks BAFF-induced MM adhesion to BMSCs. (A) MM1S and MM1R cells were incubated with BAFF (100 ng/ml) for 45 min, labeled with calcein-AM and then added to BMSC-coated 96-well plates in the presence of AMG523 or con for 2-4 h. Adherent cells were quantified by a fluorescence plate reader. (B) AMG523-inhibited adhesion induced by BAFF was performed using a 96-well plate. Data were normalized with control without BAFF or without AMG523.

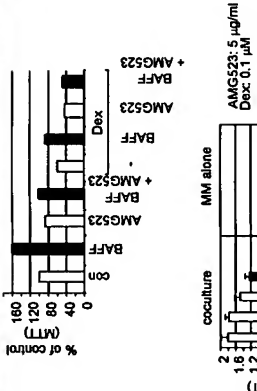


Figure 5. AMG523 blocks BAFF-induced survival. MM1S cells were incubated with BAFF (200 ng/ml) for 3 days followed by MTT assay. Data were normalized with control without BAFF or without AMG523.

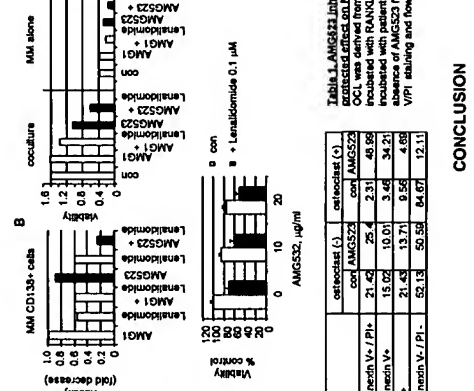


Figure 6. Enhanced anti-MM cytotoxicity of BAFF inhibitor AMG523. MM1S, INA6, and MM1R cells were incubated with AMG523 (100 ng/ml) for 3 days followed by MTT assay. Data were normalized with control without AMG523.

Figure 5. AMG523 blocks BAFF-induced survival. MM1S cells were incubated with BAFF (200 ng/ml) for 3 days followed by MTT assay. Data were normalized with control without BAFF or without AMG523.

Figure 6. Enhanced anti-MM cytotoxicity of BAFF inhibitor AMG523. MM1S, INA6, and MM1R cells were incubated with AMG523 (100 ng/ml) for 3 days followed by MTT assay. Data were normalized with control without AMG523.

Figure 7. Lenalidomide enhances AMG523-induced anti-MM cytotoxicity. MM1S cells were incubated with lenalidomide (0.5 μM) or AMG523 (10 μM) alone or together for 3 days followed by MTT assay. Data were normalized with control without lenalidomide or AMG523.

Table 1. AMG523 inhibits osteoclast (OC) activity. OCs were derived from 1-2 week cultures of PBMCs incubated with RANKL-CSF. They were then incubated with patient MM cells. In the presence or absence of AMG523, OCs were quantified by annexin V/PI staining and flow cytometric analysis.

|                  | osteoclast (+) | osteoclast (-) |
|------------------|----------------|----------------|
| % Annexin V+/PI+ | 21.42          | 25.4           |
| % Annexin V+/PI- | 15.02          | 10.01          |
| % PI+            | 21.42          | 13.71          |
| % Annexin V+/PI- | 52.13          | 50.58          |
|                  | 84.67          | 12.11          |

## CONCLUSION

The novel therapeutic AMG523 blocks the interaction between BAFF and its receptors in human MM, thereby providing the rationale for clinical trials of AMG523 to improve patient outcome in MM.

## ACKNOWLEDGEMENT

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## ORIGINAL ARTICLE

# AMG 531, a Thrombopoiesis-Stimulating Protein, for Chronic ITP

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## ABSTRACT

## BACKGROUND

Most current treatments for chronic immune thrombocytopenic purpura (ITP) act by decreasing platelet destruction. In a phase 1–2 study, we administered a thrombopoiesis-stimulating protein, AMG 531, to patients with ITP.

## METHODS

In phase 1, 24 patients who had received at least one treatment for ITP were assigned to escalating-dose cohorts of 4 patients each and given two identical doses of AMG 531 (0.2 to 10  $\mu$ g per kilogram of body weight). In phase 2, 21 patients were randomly assigned to receive six weekly subcutaneous injections of AMG 531 (1, 3, or 6  $\mu$ g per kilogram) or placebo. The primary objective was to assess the safety of AMG 531; the secondary objective was to evaluate platelet counts during and after treatment.

## RESULTS

No major adverse events that could be attributed directly to AMG 531 occurred during the treatment period; 4 of 41 patients had transient post-treatment worsening of thrombocytopenia. In phase 1, a platelet count that was within the targeted range (50,000 to 450,000 per cubic millimeter) and at least twice the baseline count was achieved in 4 of 12 patients given 3, 6, or 10  $\mu$ g of AMG 531 per kilogram. Overall, a platelet count of at least 50,000 per cubic millimeter was achieved in 7 of 12 patients, including 3 with counts exceeding 450,000 per cubic millimeter. Increases in the platelet count were dose-dependent; mean peak counts were 163,000, 309,000, and 746,000 per cubic millimeter with 3, 6, and 10  $\mu$ g of AMG 531 per kilogram, respectively. In phase 2, the targeted platelet range was achieved in 10 of 16 patients treated with 1 or 3  $\mu$ g of AMG 531 per kilogram per week for 6 weeks. Mean peak counts were 135,000, 241,000, and 81,000 per cubic millimeter in the groups that received the 1- $\mu$ g dose, the 3- $\mu$ g dose, and placebo, respectively.

## CONCLUSIONS

AMG 531 caused no major adverse events and increased platelet counts in patients with ITP. (ClinicalTrials.gov number, NCT00111475.)

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**I**MMUNE THROMBOCYTOPENIC PURPURA (ITP) is an autoimmune disorder in which antiplatelet autoantibodies cause platelet destruction.<sup>1-6</sup> The annual incidence of ITP in the United States is estimated to be approximately 16,000 cases.<sup>1,4</sup>

The initial treatment for ITP, usually corticosteroids, intravenous immune globulin, or Rh<sub>0</sub>(D) immune globulin acts primarily by interfering with platelet destruction.<sup>7</sup> Other immunomodulatory agents suppress the production of antiplatelet antibodies, but relapse is common when these agents are discontinued. Splenectomy, by contrast, can have lasting effects and even cures the disease in some patients.

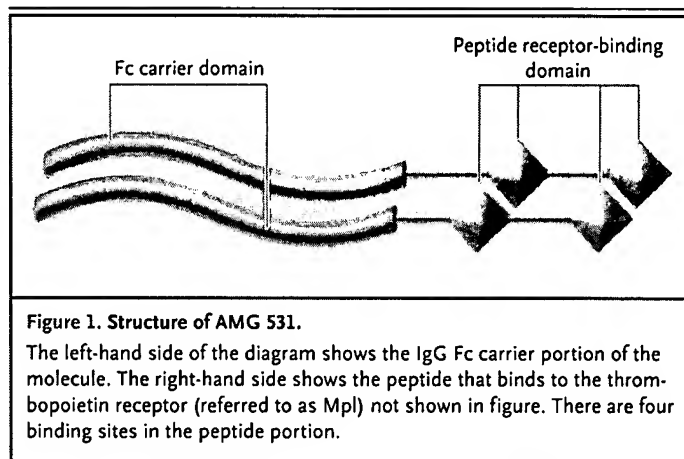
There is evidence that platelet production is suboptimal in a substantial proportion of patients with ITP,<sup>8-21</sup> suggesting that a strategy of increasing platelet production may be effective in managing the disorder. In previous studies, polyethylene glycol-conjugated recombinant human megakaryocyte growth and development factor (PEG-MGDF), a recombinant thrombopoietin, increased platelet counts in four of five patients with ITP.<sup>22,23</sup> However, antibodies against PEG-MGDF developed in healthy volunteers and in patients with cancer, and the antibodies cross-reacted with endogenous thrombopoietin in the patients, causing severe, persistent thrombocytopenia.<sup>24,25</sup> As a result, clinical development of PEG-MGDF was stopped.

A novel thrombopoiesis-stimulating protein, AMG 531 (Amgen), has recently been developed. It has no sequence homology with endogenous thrombopoietin,<sup>26,27</sup> a feature that should preclude the development of cross-reacting antibodies. We conducted a study to evaluate the safety and efficacy of treatment with AMG 531 in patients with ITP.

## METHODS

### AMG 531

AMG 531 is a protein that stimulates thrombopoiesis<sup>26</sup> (Fig. 1). It consists of disulfide-bonded human IgG1 heavy-chain and kappa light-chain constant regions (an Fc fragment) with two identical peptide sequences linked covalently at residue 228 of the heavy chain with the use of a polyglycine. The Fc component extends the half-life of the molecule in the circulation. The peptide portion was selected by screening libraries of peptides that have



**Figure 1. Structure of AMG 531.**

The left-hand side of the diagram shows the IgG Fc carrier portion of the molecule. The right-hand side shows the peptide that binds to the thrombopoietin receptor (referred to as Mpl) not shown in figure. There are four binding sites in the peptide portion.

no sequence homology with human thrombopoietin to find one with a tertiary structure that would allow it to bind to and activate the human thrombopoietin receptor, called Mpl. In healthy volunteers, AMG 531, a clear, colorless liquid administered by subcutaneous injection, increased platelet production and platelet counts and did not induce neutralizing or cross-reacting antibodies against thrombopoietin.<sup>28</sup>

### PATIENTS

Nine U.S. centers enrolled patients with chronic ITP in two sequential trials. The institutional review boards at the participating centers approved the protocols, and all patients gave written informed consent before undergoing eligibility screening. Inclusion criteria were an age of 18 to 65 years, a history of ITP (according to the American Society of Hematology guidelines<sup>9</sup>) for at least 3 months; one or more prior treatments for ITP; a mean platelet count (the mean value of two counts) of less than 30,000 per cubic millimeter (with no count >35,000 per cubic millimeter) for patients not receiving corticosteroids or a mean count of less than 50,000 per cubic millimeter (with no count >55,000 per cubic millimeter) for patients receiving corticosteroids. Patients were eligible for enrollment regardless of whether they had undergone splenectomy or whether they were receiving corticosteroid therapy, as long as there had been no changes in the corticosteroid dose or schedule of administration for at least 4 weeks. The following intervals since the last administration of therapy for ITP were required: 2 weeks for intravenous immune globulin, 8 weeks for alkylating agents, 16 weeks for rituximab, and 4 weeks

for all other treatments. Exclusion criteria were any known risk factor for thromboembolic events, a history of cardiovascular disease, active cancer, and a history of a bone marrow disorder.

#### STUDY DESIGN

This study was a multicenter, dose- and schedule-finding trial consisting of two phases (Fig. 2), with no overlap between patients in phase 1 and those in phase 2.

Phase 1 (conducted from July 1, 2002, to October 13, 2003) was an open-label, dose-escalation trial with sequential cohorts of patients. The primary objective was to assess the safety and tolerability of two injections of AMG 531 in patients with ITP. Secondary objectives were to determine the dose that would result in a platelet count that was within the targeted range (50,000 to 450,000 per cubic millimeter) and that was at least twice the baseline count and to determine the adequacy of two AMG 531 injections given within 2 to 3 weeks for achieving this range.

AMG 531 was administered to cohorts of four patients each at doses of 0.2, 0.5, 1, 3, 6, and 10  $\mu$ g per kilogram of body weight. The drug was administered by subcutaneous injection on day 1, followed by 14 days of observation. Health status, complete blood counts, and blood chemical values were monitored throughout the study. Assays to detect anti-AMG 531 antibodies were performed before treatment, at the end of treatment, and at the end of the study. If the platelet count was less than 50,000 per cubic millimeter on day 15, a second identical dose was administered. If the platelet count was 50,000 per cubic millimeter or more on day 15, the second dose was delayed until day 22; if at that time the platelet count remained 50,000 per cubic millimeter or more, the second dose was not given. A data review committee, composed of investigators and Amgen staff members who were not directly involved in the execution of the study, reviewed antibody and other safety data from the current and previous cohorts before treating the next sequential cohort. The protocol required termination of the study if antibodies that cross-reacted with thrombopoietin were detected. After the treatment was completed, patients were followed for an additional 8 weeks.

Phase 2 (conducted from October 6, 2003, to June 17, 2004) was a double-blind, placebo-controlled evaluation of AMG 531 in patients with ITP. The placebo consisted of the excipients of the

AMG 531 formulation. The objectives were to evaluate the safety of AMG 531 and to determine a weekly dose that would result in a platelet count that was within the targeted range used in phase 1 (50,000 to 450,000 per cubic millimeter) and that was at least twice the baseline count. Patients were randomly assigned to receive AMG 531 at one of three doses (1, 3, or 6  $\mu$ g per kilogram) or placebo once a week for 6 weeks, with a ratio of AMG 531 assignments to placebo assignments of 4:1. A protocol amendment later eliminated the 6- $\mu$ g cohort; only one patient was randomly assigned to this dose. No dose adjustments were allowed, although doses were withheld in the event of a platelet count greater than 350,000 per cubic millimeter. Patients were followed for 6 weeks after the last dose of study drug.

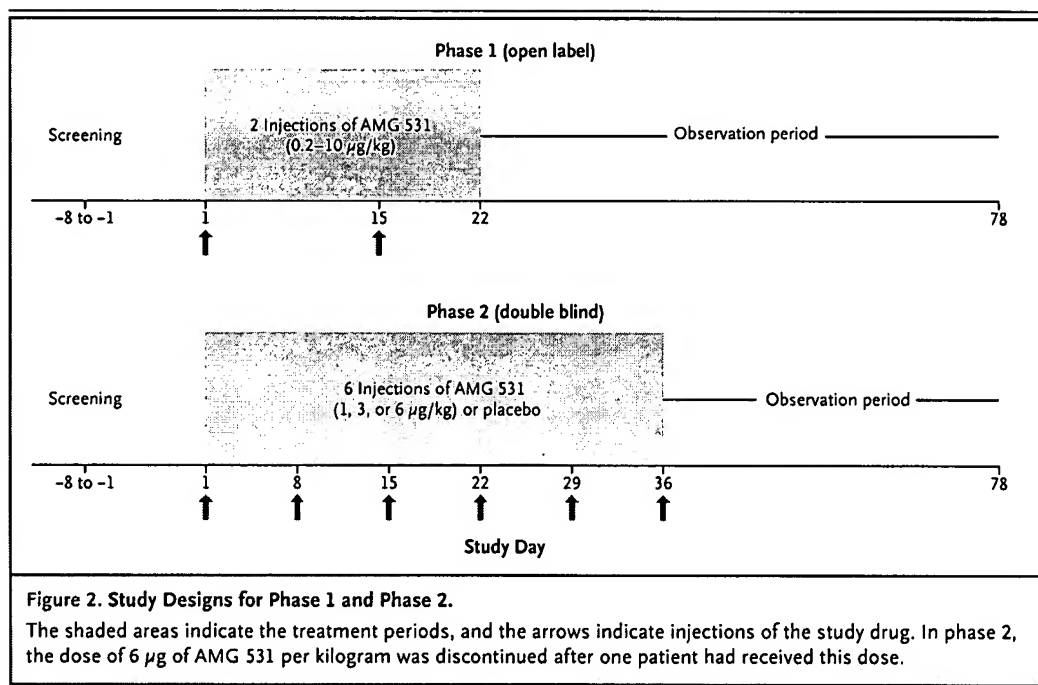
In collaboration with the investigators, Amgen designed the study, conducted the statistical analyses, and interpreted the data, which Amgen held. The investigators had unrestricted access to the primary data. The academic authors wrote this article, with editing assistance from Amgen; no limitations were imposed by Amgen in the writing of the article. The authors vouch for the completeness and accuracy of the results.

#### ASSAYS FOR THROMBOPOIETIN AND FOR ANTIBODIES AGAINST AMG 531 AND THROMBOPOIETIN

Blood samples were drawn at baseline and at specified points under standard conditions. The serum was separated, frozen, and shipped to Amgen for testing. Thrombopoietin levels were measured with the use of a modified, commercially available enzyme-linked immunosorbent assay kit (R&D Systems).<sup>27</sup> The presence of antibodies that neutralized AMG 531 or thrombopoietin was determined with the use of a cell-based bioassay.<sup>27,28</sup> The threshold of detection was approximately 400 ng per milliliter for a control rabbit antihuman AMG 531 antibody and 200 ng per milliliter for a control rabbit antihuman thrombopoietin antibody.

#### STATISTICAL ANALYSIS

Demographic characteristics, baseline clinical characteristics, and hematologic and other laboratory values were summarized with the use of descriptive statistics. Enrolled patients were included in all analyses, except that the single patient randomly assigned to the 6- $\mu$ g dose of AMG 531 in phase 2 was excluded from the platelet-related analyses. The study did not have the statistical power to de-



tect a significant difference between dose groups, and no statistical hypothesis testing was performed.

A general linear model was used to investigate the relationship between the peak platelet count and the baseline thrombopoietin level, adjusted for the AMG 531 dose received. Logistic-regression models, adjusted for the AMG 531 dose, were used to determine whether the platelet response was associated with any of the following variables: the baseline serum thrombopoietin level, the baseline platelet count, splenectomy status, and the presence or absence of concurrent corticosteroid therapy.

Platelet counts obtained after the administration of rescue medications were excluded from the efficacy analyses. A rescue medication was defined as any medication, not administered at baseline, that was given to increase platelet counts, or corticosteroids given at a higher dose or frequency than that at baseline.

## RESULTS

### STUDY POPULATIONS

#### Phase 1

A total of 24 patients with ITP were enrolled in six dose cohorts (4 patients each at AMG 531 dose levels of 0.2, 0.5, 1, 3, 6, and 10 µg per kilogram).

Table 1 summarizes the demographic and clinical characteristics of the patients at baseline. The majority of the patients were women (17 of 24) and were white (22 of 24); the median age was 45 years. The median platelet count at baseline was 11,000 per cubic millimeter, and the median time since the diagnosis of ITP was 6.2 years. Seven patients (29%) were receiving corticosteroids, and 19 (79%) had undergone a splenectomy.

#### Phase 2

A total of 21 patients were enrolled in phase 2; 4 were assigned to the placebo group, and 17 to one of the AMG 531 groups (8 each to the 1-µg and 3-µg groups and 1 to the 6-µg group). The placebo and AMG 531 groups were similar with respect to demographic characteristics (Table 1). Fifteen patients were women, and 14 were white; the median age was 49 years. The median platelet count at baseline was 16,000 per cubic millimeter, and the median time since the diagnosis of ITP was 5.2 years. Seven patients (33%) were receiving corticosteroids, and 14 (67%) had undergone a splenectomy.

### SAFETY

Table 2 summarizes adverse events that occurred in 10% or more of the patients. Adverse events with an incidence of less than 10% are provided in the

Table 1. Baseline Characteristics of the Patients.\*

| Characteristic                        | Phase 1                             |                                    | Phase 2                        |                                |  |
|---------------------------------------|-------------------------------------|------------------------------------|--------------------------------|--------------------------------|--|
|                                       | AMG 531, 0.2–1 $\mu$ g/kg<br>(N=12) | AMG 531, 3–10 $\mu$ g/kg<br>(N=12) | AMG 531, 1 $\mu$ g/kg<br>(N=8) | AMG 531, 3 $\mu$ g/kg<br>(N=8) | AMG 531, 6 $\mu$ g/kg<br>(N=1)<br>Placebo<br>(N=4) |
| Sex — no. (%)                         |                                     |                                    |                                |                                |  |
| Female                                | 8 (67)                              | 9 (75)                             | 6 (75)                         | 5 (62)                         | 3 (75)   |
| Male                                  | 4 (33)                              | 3 (25)                             | 2 (25)                         | 3 (38)                         | 1 (25)   |
| Race — no. (%)†                       |                                     |                                    |                                |                                |  |
| White                                 | 12 (100)                            | 10 (83)                            | 5 (62)                         | 5 (62)                         | 3 (75)   |
| Black                                 | 0                                   | 2 (17)                             | 1 (12)                         | 0                              | 0  |
| Other                                 | 0                                   | 0                                  | 2 (25)                         | 3 (38)                         | 1 (25)   |
| Age — yr                              |                                     |                                    |                                |                                |  |
| Median                                | 45                                  | 47                                 | 45                             | 53                             | 42   |
| Range                                 | 26–60                               | 21–65                              | 20–63                          | 19–62                          | —  |
| Weight — kg                           |                                     |                                    |                                |                                |  |
| Median                                | 80                                  | 102                                | 73                             | 79                             | 88   |
| Range                                 | 55–176                              | 57–135                             | 59–112                         | 57–86                          | 68–110   |
| Platelets — per mm <sup>3</sup>       |                                     |                                    |                                |                                |  |
| Median                                | 9000                                | 12,000                             | 17,000                         | 12,000                         | 15,000   |
| Range                                 | 4000–31,000                         | 5000–27,000                        | 4000–25,000                    | 5000–23,000                    | 6000–49,000  |
| Thrombopoietin — pg/ml                |                                     |                                    |                                |                                |  |
| Median                                | 75                                  | 62                                 | 92                             | 105                            | 110  |
| Range                                 | 31–135                              | 30–173                             | 68–185                         | 47–118                         | —  |
| Time since ITP diagnosis — yr         |                                     |                                    |                                |                                |  |
| Median                                | 7.1                                 | 4.7                                | 5.6                            | 9.1                            | 6.4  |
| Range                                 | 1.2–44.4                            | 1.2–19.5                           | 0.5–24.9                       | 0.4–37.0                       | —  |
| Concomitant corticosteroids — no. (%) | 2 (17)                              | 5 (42)                             | 1 (12)                         | 3 (38)                         | 0  |
| Dose — mg/day                         |                                     |                                    |                                |                                |  |
| Median                                | 3.8                                 | 10.0                               | 7.5                            | 20.0                           | —  |
| Range                                 | 2.5–5.0                             | 5.0–40.0                           | —                              | 2.5–20.0                       | —  |

|  |          |          |          |          |         |        |
|--|----------|----------|----------|----------|---------|--------|
| No concomitant corticosteroids — no. (%) | 10 (83)  | 7 (58)   | 7 (88)   | 5 (62)   | 1 (100) | 1 (25) |
| Previous ITP therapies — no. (%)         |          |          |          |          |         |        |
| 1–3                                      | 5 (42)   | 4 (33)   | 3 (38)   | 2 (25)   | 0       | 1 (25) |
| 4–6                                      | 6 (50)   | 6 (50)   | 5 (62)   | 3 (38)   | 1 (100) | 3 (75) |
| >6                                       | 1 (8)    | 2 (17)   | 0        | 3 (38)   | 0       | 0      |
| Splenectomy — no. (%)                    | 11 (92)  | 8 (67)   | 5 (62)   | 7 (88)   | 1 (100) | 1 (25) |
| Time since splenectomy — yr              |          |          |          |          |         |        |
| Median                                   | 7.3      | 5.4      | 5.8      | 10.9     | 5.4     | 0.3    |
| Range                                    | 0.8–44.4 | 1.3–18.5 | 0.5–24.5 | 0.6–37.0 | —       | —      |
| No splenectomy — no. (%)                 | 1 (8)    | 4 (33)   | 3 (38)   | 1 (12)   | 0       | 3 (75) |

\* In phase 1, AMG 531 was administered twice, 2 weeks apart; in phase 2, it was administered weekly for 6 weeks.

† Race was self-reported.

Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

#### Phase 1

Since single doses of AMG 531 that were less than 3  $\mu$ g per kilogram did not affect the platelet count in phase 1, data on adverse events were considered for two combined dose groups: 0.2 to 1  $\mu$ g per kilogram and 3 to 10  $\mu$ g per kilogram. The most frequently reported adverse events were contusions, ecchymosis, or both, which occurred in 67% of the 24 patients (6 of 12 patients receiving 0.2 to 1  $\mu$ g per kilogram and 10 of 12 receiving 3 to 10  $\mu$ g per kilogram), and mild-to-moderate headache, which occurred in 46% of the patients (6 of 12 receiving 0.2 to 1  $\mu$ g per kilogram and 5 of 12 receiving 3 to 10  $\mu$ g per kilogram).

Serious adverse events were reported in three patients. One patient receiving the 0.2- $\mu$ g dose had grade 3 vertigo, thought by the investigator to be unrelated to AMG 531, and was briefly hospitalized. The second patient, also receiving the 0.2- $\mu$ g dose, had a life-threatening subdural hemorrhage 21 days after the second injection, which was also thought by the investigator to be unrelated to AMG 531. In the third patient, who received the 10- $\mu$ g dose, the platelet count transiently decreased below the baseline value after the discontinuation of treatment; this event was thought by the investigator to be related to the withdrawal of AMG 531.

#### Phase 2

The most frequently reported adverse events in phase 2 were contusions, ecchymosis, or both (occurring in 59% of the patients in the AMG 531 groups and 75% of those in the placebo group); epistaxis (41% and 50%, respectively); mild-to-moderate headache (29% and 0%, respectively), and oral mucosal blistering (also 29% and 0%, respectively) (Table 2). All patients with oral mucosal blistering had a history of oral bleeding and had bleeding at the time of enrollment. Most bleeding events occurred during the post-treatment period or in patients who did not have a response to the study drug.

Three patients (two who received placebo and one who received AMG 531) had serious adverse events. The two patients in the placebo group had a total of three serious adverse events: one had asthma, and the other had an intracranial hemorrhage and, after splenectomy, popliteal deep-vein

Table 2. Adverse Events in 10% or More of Patients.\*

| Adverse Event   | Phase 1                             |                                    | Phase 2                              |                  | Total                        |
|---|-------------------------------------|------------------------------------|--------------------------------------|------------------|------------------------------|
|   | AMG 531, 0.2–1 $\mu$ g/kg<br>(N=12) | AMG 531, 3–10 $\mu$ g/kg<br>(N=12) | AMG 531, 1 to 6 $\mu$ g/kg<br>(N=17) | Placebo<br>(N=4) | AMG 531, all doses<br>(N=41) |
|   | number (percent)                    |                                    |                                      |                  |                              |
| Contusions, ecchymosis,<br>or both                                  | 6 (50)                              | 10 (83)                            | 10 (59)                              | 3 (75)           | 26 (63)                      |
| Headache  | 6 (50)                              | 5 (42)                             | 5 (29)                               | 0                | 16 (39)                      |
| Petechiae   | 3 (25)                              | 5 (42)                             | 4 (24)                               | 1 (25)           | 12 (29)                      |
| Epistaxis   | 1 (8)                               | 2 (17)                             | 7 (41)                               | 2 (50)           | 10 (24)                      |
| Fatigue   | 5 (42)                              | 3 (25)                             | 1 (6)                                | 0                | 9 (22)                       |
| Oral mucosal blistering   | 1 (8)                               | 2 (17)                             | 5 (29)                               | 0                | 8 (20)                       |
| Gingival bleeding   | 1 (8)                               | 2 (17)                             | 4 (24)                               | 1 (25)           | 7 (17)                       |
| Dizziness   | 1 (8)                               | 3 (25)                             | 2 (12)                               | 1 (25)           | 6 (15)                       |
| Upper respiratory tract in-<br>fection (not otherwise<br>specified) | 4 (33)                              | 2 (17)                             | 0                                    | 0                | 6 (15)                       |
| Excoriation   | 1 (8)                               | 1 (8)                              | 3 (18)                               | 0                | 5 (12)                       |
| Nausea  | 3 (25)                              | 0                                  | 2 (12)                               | 1 (25)           | 5 (12)                       |
| Arthralgia  | 3 (25)                              | 1 (8)                              | 0                                    | 1 (25)           | 4 (10)                       |
| Peripheral edema  | 1 (8)                               | 2 (17)                             | 1 (6)                                | 0                | 4 (10)                       |
| Rash (not otherwise<br>specified)                                   | 3 (25)                              | 1 (8)                              | 0                                    | 0                | 4 (10)                       |
| Worsening of thrombocyto-<br>penia                                  | 0                                   | 1 (8)                              | 3 (18)                               | 0                | 4 (10)                       |

\* In phase 1, AMG 531 was administered twice, 2 weeks apart; in phase 2, it was administered weekly for 6 weeks. Adverse events that occurred in less than 10% of the patients are listed in the Supplementary Appendix (available with the full text of this article at [www.nejm.org](http://www.nejm.org)).

thrombosis. The patient treated with AMG 531 (3  $\mu$ g per kilogram) had vaginal bleeding with severe but transient worsening of thrombocytopenia 19 days after the discontinuation of AMG 531.

None of the patients had a positive test for antibodies against AMG 531 or thrombopoietin. With the exception of platelet counts, no clinically significant changes were observed in vital signs or in hematologic or serum chemical values.

#### EFFICACY

##### Phase 1

The targeted platelet response was not achieved in the patients who received 0.2, 0.5, or 1  $\mu$ g of AMG 531 per kilogram, with the exception of one patient in the 0.2- $\mu$ g group, who had received rituximab 4 weeks before enrollment. Of the 12 patients who received AMG 531 at a dose of 3, 6, or 10  $\mu$ g per kilogram, however, 4 had platelet counts that reached the targeted range, and 3 additional patients had counts that exceeded 450,000 per cubic millimeter, for a total of 7 patients with

peak platelet counts that were 50,000 per cubic millimeter or higher (Fig. 3). The median time to the targeted response ranged from 5 to 8 days. The increase in platelet counts appeared to be dose-dependent. Mean peak platelet counts after the first AMG 531 injection were 163,000 per cubic millimeter for 3  $\mu$ g per kilogram, 309,000 per cubic millimeter for 6  $\mu$ g per kilogram, and 746,000 per cubic millimeter for 10  $\mu$ g per kilogram. Median times to peak counts were 11, 10, and 14 days, respectively. Individual peak platelet counts were highly variable at each dose level.

##### Phase 2

In one patient, the platelet count increased to 520,000 per cubic millimeter on day 21. The data review committee closed the highest-dose cohort because of concern that the patient had received the 6- $\mu$ g dose and that the platelet count exceeded the upper limit of the targeted range. When the study was unblinded, it was confirmed that the patient had received the 6- $\mu$ g dose.

Weekly doses of AMG 531 at 1 and 3  $\mu$ g per



kilogram increased the platelet count in most patients (Fig. 4). The median time from the first dose to the peak count was 18 days (range, 8 to 43) for 1  $\mu\text{g}$  per kilogram, 19 days (range, 8 to 36) for 3  $\mu\text{g}$  per kilogram, and 63 days (range, 7 to 78) for placebo. The targeted platelet range was reached in seven of eight patients receiving 1  $\mu\text{g}$  per kilogram and in three of eight patients receiving 3  $\mu\text{g}$  per kilogram; the targeted range was exceeded in an additional two patients receiving the 3- $\mu\text{g}$  dose. Overall, in 12 of 16 patients treated with AMG 531 at a weekly dose of 1 or 3  $\mu\text{g}$  per kilogram, the targeted range was reached (in 10 patients) or exceeded (in 2 patients), and in 9 of these patients, the platelet response had occurred by the first assessment on day 8.

In five patients at each dose (1 and 3  $\mu\text{g}$  per kilogram), the peak platelet count exceeded 100,000 per cubic millimeter. A total of 14 patients treated with AMG 531 had increases in platelet counts of at least 20,000 per cubic millimeter over the baseline count. Individual platelet responses varied in the AMG 531 and placebo groups (Fig. 1 of the Supplementary Appendix). The mean peak platelet counts were 135,000 and 241,000 per cubic millimeter in the groups that received 1 and 3  $\mu\text{g}$  per kilogram, respectively, and 81,000 per cubic millimeter in the placebo group; these counts were 8.5, 17, and 2.7 times as high as the baseline counts, respectively. One of four patients in the placebo group had a spontaneous remission; this patient had undergone a splenectomy 3.5 months before entering the study.

No significant relationship was observed between the peak platelet count and the baseline thrombopoietin level. Only the baseline platelet count in phase 1 was predictive of the platelet response (the higher the baseline count, the greater the likelihood of a platelet response;  $P=0.049$ ).

## DISCUSSION

Thrombocytopenia occurs in patients with ITP when the rate of platelet destruction exceeds the ability of the bone marrow to increase platelet production. Autoantibodies from patients with ITP inhibit the production of megakaryocytes in vitro.<sup>19,20</sup> In addition, apoptotic megakaryocytes have been detected in such patients by means of electron microscopy.<sup>19-21</sup> Moreover, kinetic data show that platelet production, as measured by platelet turnover, is reduced or normal in approximately two thirds of patients with ITP.<sup>16-18</sup> Plasma levels

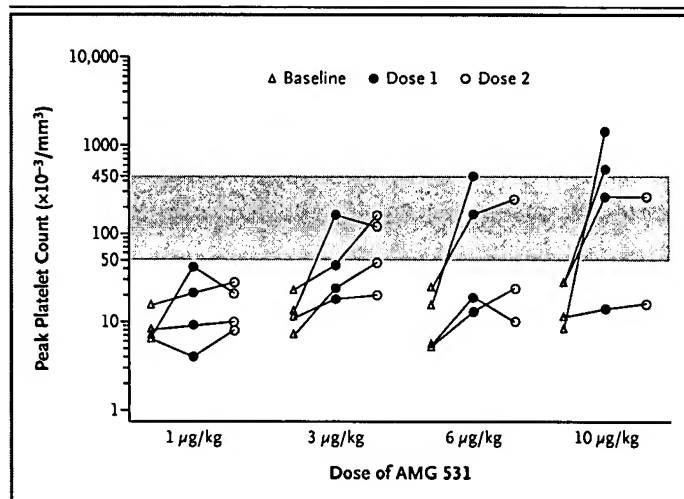


Figure 3. Peak Individual Platelet Counts in Phase 1.

The baseline platelet count and the peak platelet count after dose 1 and dose 2 are shown. There were four patients in each dose cohort. Three patients did not receive a second dose. The shaded area shows the targeted platelet range. Platelet counts associated with the use of rescue medication have been excluded.

of endogenous thrombopoietin are not elevated in patients with ITP, as they are in patients with thrombocytopenia due to chemotherapy or aplastic anemia<sup>8-15</sup>; the mechanism underlying this phenomenon is thought to be related to clearance of endogenous thrombopoietin by the megakaryocyte and platelet pools.<sup>29,30</sup> All these findings, which suggest that platelet production in ITP is often impaired, led to the hypothesis that stimulation of platelet production can alleviate the thrombocytopenia.

We evaluated AMG 531 in 41 patients with severe, refractory ITP; 32 of the patients had had no response to conventional treatments, including splenectomy. None of the patients had neutralizing antibodies to AMG 531 or thrombopoietin after as many as six weekly doses of AMG 531. Except for headache and transient post-treatment worsening of thrombocytopenia, all adverse events appeared to be related to the underlying disease. Thirty-nine percent of patients reported mild-to-moderate headache, occurring within 24 hours after the administration of AMG 531 and persisting for several hours, which was controlled with acetaminophen and did not require the discontinuation of treatment.

AMG 531 does not appear to affect the ongoing rate of platelet destruction; in all patients receiving the drug, the platelet count returned to the

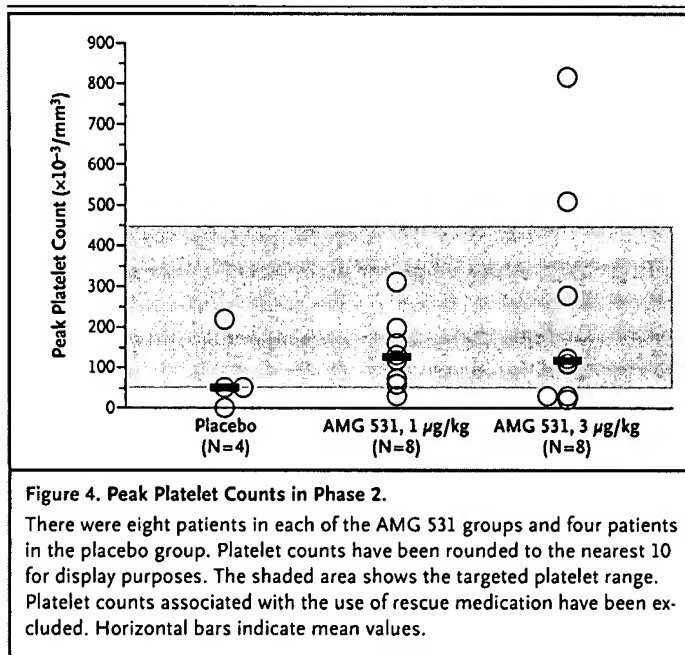


Figure 4. Peak Platelet Counts in Phase 2.

There were eight patients in each of the AMG 531 groups and four patients in the placebo group. Platelet counts have been rounded to the nearest 10 for display purposes. The shaded area shows the targeted platelet range. Platelet counts associated with the use of rescue medication have been excluded. Horizontal bars indicate mean values.

baseline value after the discontinuation of short-term treatment. In four patients with a platelet response, at least one post-treatment count was less than 10,000 per cubic millimeter and was at least 10,000 per cubic millimeter lower than the baseline value within 4 weeks after the last dose; these low counts persisted for 3 to 17 days. In two of the four patients, rescue treatment with intravenous immune globulin or corticosteroids was administered and was successful. This transient post-treatment worsening of thrombocytopenia had no relationship to the peak platelet count or any other clinical variable that we could evaluate. The phenomenon may be due to increased clearance of endogenous thrombopoietin by the pharmacologically expanded megakaryocyte mass, as demonstrated in studies of animals and humans.<sup>29,31</sup>

Patients completing this and other AMG 531 studies were eligible to enroll in an ongoing, open-label extension with weekly administration of AMG 531 and dose adjustment based on the platelet count. Preliminary data<sup>32</sup> indicate that 21 of 26 patients treated for up to 24 weeks had a protocol-defined platelet response to AMG 531 and 12 had a durable response. Two patients who participated in the present study and subsequently entered the extension study were found to have a mild-to-moderate increase in bone marrow reticulin but without collagen fibrosis and with

normal cytogenetic findings.<sup>33</sup> Both patients were asplenic and were receiving relatively high doses of AMG 531 (>10 µg per kilogram), with a minimal response or none. A bone marrow biopsy performed in one of the patients 14 weeks after discontinuation of the treatment showed decreased reticulin deposition. A follow-up biopsy specimen has not yet been obtained for the other patient. In animal models, the increase in reticulin with the administration of PEG-MGDF resolved after discontinuation of the drug,<sup>34,35</sup> and in patients with leukemia who were treated with thrombopoietin, the increase resolved within 6 weeks after the discontinuation of treatment.<sup>36</sup>

In our study, with the results combined for doses higher than 1 µg per kilogram in phase 1 and for the 1-µg and 3-µg doses in phase 2, 19 of 28 patients had platelet counts that reached or exceeded the targeted range. No complications, including thrombotic events, were reported for patients with platelet counts that exceeded 450,000 per cubic millimeter. In phase 2, considerable week-to-week fluctuation in platelet counts was observed, even though the administered dose of AMG 531 was not changed. The role of individual dose adjustment will need to be explored in future studies. The mechanism by which AMG 531 increases platelet counts in ITP may involve the prevention of premature megakaryocyte apoptosis, as well as stimulation of megakaryocyte progenitors and megakaryocyte maturation and endomitosis, as has been demonstrated with other thrombopoietic growth factors.<sup>37</sup>

In this dose-finding study, not all patients received the optimal dose of AMG 531 or the optimal schedule of administration. For this reason and because of the short duration of treatment in a relatively small number of patients, the results must be considered preliminary.

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reports having an equity interest in Amgen. No other potential conflict of interest relevant to this article was reported.

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**CORRECTION**

**AMG 531, a Thrombopoiesis-Stimulating Protein, for Chronic ITP**

AMG 531, a Thrombopoiesis-Stimulating Protein, for Chronic ITP . On page 1672, the ninth line of the Results section of the Abstract should have read "3, 6, and 10  $\mu$ g of AMG 531 per kilogram," rather than "3, 6, and 10  $\mu$ g of AMG 531 per cubic millimeter," as printed. The text has been corrected on the *Journal's* Web site at [www.nejm.org](http://www.nejm.org). We regret the error.